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A METHOD OF DETERMINING ALLERGENIC PROPERTIES OF CHEMICAL SUBSTANCES

(K metodike opredeleniya allergennykh svoystv khimicheskikh veshchestv)

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A METHOD OF DETERMINING ALLERGENIC PROPERTIES OF CHEMICAL SUBSTANCES

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The intense chemization of industry, agriculture and medicine and the wide use of chemical substances in everyday life attaches a special urgency to developing reliable, technically simple and quick methods of determining the allergenic properties of various chemical products. In past years, many researchers have used multiple intradermal injections of the supposed allergen in guinea pigs. However, because allergy to chemical substances in man most often appears clinically in the form of dermatoses, in recent years the more adequate method of epicutaneous applications has been chosen. Variants of this method are many: the thirty drop applications (Ye. A. Ievleva) or the 1-4 compress applications (Buehler) on one section of the lateral surface of the trunk; single application of various cultivations of the substance on 8 sections of the body (N. M. Zalkan); applications to the back of the neck for 2 weeks (Hunziker) or once (Weck and Frey); 3 applications to the ear (Stevens), and so forth.

We shall not dwell on methods of experimental sensitization of man, as this means of solving the problem, for completely understandable reasons, has not been widely used. The most suitable object remains a white guinea pig, as its allergic reactivity is the closest to man's. Analysis of different variants of epicutaneous sensitization leads to the conclusion that 1-3 applications, even to the most reactive zone of the skin (ear, back of the neck) sensitizes only with the use of very strong allergens. At the same time, very long application schedules are also unsuitable, because they can cause the development of tolerance (Hunziker, Fettich). The optimum epicutaneous sensitization regimen must reduce to minimum the primary, obligatory injury to the skin, symptoms of intoxication must not develop; however, the incorporated

dose must be above threshold even when a product which does not penetrate the skin easily is being used. Selecting an optimum scheme by varying the number of applications and concentrations of substances is usually prolonged. The method of epicutaneous application is also inadequate for allergization of the organism through respiratory organs or the gastro-intestinal tract.

It is perfectly clear that toxicological practice requires a universal method to determine the allergic activity of chemical products, regardless of the "entrance gate" of the allergen. Such a method does exist in allergology. We mean the injection of 0.5-1 mg of the substance mixed with Freund adjuvant into the pad of the paws of the guinea pigs. However, many chemical allergens in such dosages are toxic (and intoxication inhibits the development of allergic reactions); technically the method is also unsuitable (necessity to heat the mixture, difficulty in measuring when working with an emulsion, etc.). In connection with this, we used a single intradermal injection of the allergen to the ear of the guinea pig. Here it is possible to manage without the Freund stimulant and significantly reduce the dosage. According to the data of Stevens, the skin of the ear is very sensitive to drop applications; Macher and Chase, studying the mechanism of sensitization and toleration with regard to extremely strong allergens DNCB and picrylchloride, found that injecting 0.25-0.5 μg of these substances into the skin of the ear sensitizes some guinea pigs.

Tests were conducted on guinea pigs weighing 250-300 g; a tuberculin syringe was used to inject into the skin of the outer surface of the ear 0.02 ml of the allergen solution, diluted with a physiological solution or acetone in ratios of 1:500, 1:1000, 1:2000 and 1:4000. The animals received approximately 5 to 50 μg of the chemical substances. Control animals received one solvent. Preliminary tests showed that the allergy can best be shown by skin drop tests 11-12 days after the sensitizing injection (allergy is shown on the 7th day when weak allergens are used only in individual animals). Skin tests with 1 drop of a minimal solution of the test-allergen, causing no visible reaction in the control animals, were made strictly in the middle section of the lateral surface of the trunk and evaluated in points.

We used a universal scale to evaluate reactions to allergens of practically any strength: 0--no visible reaction; 1--weak focal erythema; 2--

strong focal erythema; 3--moderate generalized or diffuse focal erythema; 4--strong generalized erythema; 5--moderate or strong erythema with thickening of skin folds; 6--strong erythema, considerable thickening of skin, focal hemorrhage or ulceration (A. S. Raben et al.).

This method was used to test 6 chemical products, previously studied by the method of epicutaneous applications. The most allergenic was epoxy furfuryl ether (EF). Moderate allergenic properties were shown by epoxidized triethyleneglycol (TEG-1) and complex products--fiberglass lubricant No. 30 and PE. Weak allergenic properties are characteristic of dicyanamideformaldehyde acetic resin (DCA) and epichlorohydrin (ECH).

Unlike tests with epicutaneous sensitization, in this case the least active was lubricant No. 30. Evidently, lubricant PE and especially DCA do not easily penetrate the skin, which maximized their allergenic activity in epicutaneous sensitization.

Two of these allergens--EF and ECH--were dissolved only in acetone. However, acetone is not indifferent for tissues; in the control animals, into the skin of the ear of which were injected 0.02 ml of acetone, an intense inflammatory reaction developed, often ending in 12-13 days by perforation of the ear. This inflammatory process considerably increased the sensitivity of the animals to the first skin irritating properties of the epoxides. In this connection, dilution of the test-allergen could be increased 100 times for EF and 10 times for ECH. However, in this case as well, both, especially EF, were strong allergens (sensitization of all animals occurred with dilution of the allergen 1:4000). Reducing the concentration of the weaker allergen TEG-1 10 times in skin tests weakened the intensity of the allergy in comparison with the use of a physiological solution.

On the basis of epicutaneous sensitization, the tested products could be arranged in the following order, according to their allergenic activity: EF>TEG-1>lubricant No. 30>lubricant PE>DCA>ECH. However, this order characterizes only their ability to allergize the organism through undamaged skin, which is a rather strong mechanical barrier. True allergenic properties, appearing in tests when a small number of products were injected into the skin of the ear, were different: EF>ECH>TEG-1>lubricant PE>DCA>lubricant No. 30.

This order corresponds better to the level of allergic diseases of people who come in contact with these substances, which is completely natural, as in industry the chemical substances enter the organism not only through the skin, but also by inhalation.

The method we have suggested of determining the allergenic properties of chemical substances does not require long tests (11-12 days), it makes it possible to find the allergenic properties of weak sensitizers, it is technically simple, it can be used when the studied substance is not very soluble (dilution not less than 1:500), and, judging by the tests with acetone solutions, is practically unlimited by the character of the solvent. It is necessary only to first select the dilution of the test-allergen on animals which have received 0.02 ml of the solvent for 11-12 days in the skin of the ear.

The dilution of the allergen used in sensitization can serve as a characteristic of its allergenic activity. In our tests, strong allergens (EF, ECH) sensitized with complete reliability in dilutions of 1:4000-1:2000, moderate (TEG-1, lubricant PE)--in dilutions of 1:1000 and weak (DCA, lubricant No. 30) --in dilutions of 1:500. Judging by the data of Macher and Chase, extremely strong allergens can sensitize in dilutions greater than 1:10,000. If several allergens are equal according to sensitizing dosage (for example, DCA and lubricant No. 30; TEG-1 and lubricant PE; EF and ECH)then a detailed comparison can be made according to the dilution of the test- allergen and the average point value (EF in dilution of 1:1000 caused a reaction with an average point value of 3.5-3.3, and ECH in dilution of 1:20 caused an average point value of 2.7).

Conclusions

1. A single injection into the skin of the ear of 0.02 ml of dilutions of all 6 tested allergens (from 1:500 and above) sensitized guinea pigs in 11-12 days.

2. The allergenic activity of chemical substances can be compared by their dilution in the sensitizing injection.

3. As the suggested method is based on the injection of large dilutions of the tested substances, it can be used to study poorly soluble products and a small amount of the injected allergen (weighing not more than 40 μg) guar-

antees against intoxication, which weakens the development of allergy.

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